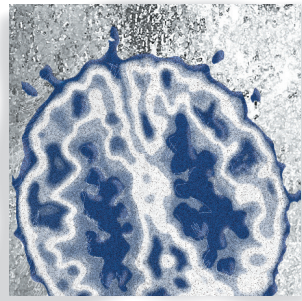# Biological markers of generalized anxiety disorder

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Generalized anxiety disorder (GAD) is a prevalent and highly disabling mental health condition; however, there is still much to learn with regard to pertinent biomarkers, as well as diagnosis, made more difficult by the marked and common overlap of GAD with affective and anxiety disorders. Recently, intensive research efforts have focused on GAD, applying neuroimaging, genetic, and blood-based approaches toward discovery of pathogenetic and treatment-related biomarkers. In this paper, we review the large amount of available data, and we focus in particular on evidence from neuroimaging, genetic, and neurochemical measurements in GAD in order to better understand potential biomarkers involved in its etiology and treatment. Overall, the majority of these studies have produced results that are solitary findings, sometimes inconsistent and not clearly replicable. For these reasons, they have not yet been translated into clinical practice. Therefore, further research efforts are needed to distinguish GAD from other mental disorders and to provide new biological insights into its pathogenesis and treatment.

Introduction

Generalized anxiety disorder (GAD) is a serious psychiatric condition, affecting up to 6% of the population during their lifetime[1]; if not appropriately treated, it has a chronic course and carries a high burden of disability and public burden. Its manifestation is complicated by the comorbidity with other psychiatric disorders, such as major depressive disorder (MDD), panic disorder, and alcohol/substance abuse,[2] which additionally aggravate outcome and contribute to a poor treatment response. Patients with GAD are frequently users of primary care resources in Western countries, having a large impact on the health care system.[3] As with treatment of other psychiatric disorders, the treatment of GAD involves two targets—a reduction in acute symptoms and relapse prevention in the long term.[4] Until now, international guidelines for GAD treatment have recommended selective serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs), and pregabalin as first-line options, owing to their established efficacy and good safety profiles, with benzodiazepines such as diazepam as second-line options.[5] However, delayed action, worsening of anxious symptoms in the first

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days of treatment, and troublesome side effects—such as nausea and sexual dysfunction for SSRIs and SNRIs and dizziness and sedation for pregabalin—are often reasons of treatment discontinuation and lack of desirable therapeutic outcome.[4] In addition, a large proportion of GAD patients do not obtain a sufficient response to first-line treatment or they continue to have residual symptoms; they, therefore, are consequently at high risk of experiencing disorder chronicity and a low quality of life.[6]

Because of these unmet needs, attempts at new pharmacological approaches for the treatment of GAD have been introduced, for example, mood stabilizers and atypical antipsychotics in monotherapy or in augmentation of standard treatment with SSRIs/SNRIs.[4] However, no other agents—including quetiapine, which shows the most robust anxiolytic effect among antipsychotics—can be recommended, at least not as first-line options for GAD treatment. Unfortunately, recent attempts to find new targets for GAD treatment—in particular, corticotropin-releasing factor (CRF) receptors—were not successful.

GAD often remains marginalized and neglected due to traditional diagnostic conception; there is a continuous debate between researchers and clinicians about its nosological and neurobiological uniqueness.[7] However, the one approach that is certain to distinguish GAD from other mental health disorders and to improve understanding of GAD phenomenology is the investigation of biological factors commonly referred to as biomarkers that underlie GAD pathogenesis and treatment outcomes. Psychiatric research efforts have recently prioritized such identification of biomarkers as it may significantly improve earlier diagnosis and prevention strategies for mental disorders. GAD has recently become the focus of intensive research efforts applying neuroimaging and genetic approaches toward discovery of the pathogenetic biomarkers for GAD; however, only a few studies have specifically addressed predictors of treatment response. In this paper, we review the large amount of available data and focus in particular on evidence from neuroimaging, genetic, and neurochemical measurements in GAD in order to better understand potential biomarkers involved in its etiology and treatment.

Neuroimaging biomarkers

To date, the main evidence for biomarkers in GAD is derived from neuroimaging studies, including those using structural (magnetic resonance imaging [MRI], diffusion tensor imaging [DTI]), functional (functional MRI [fMRI], positron emission tomography [PET]/single-photon emission computed tomography [SPECT]), and biochemical (proton magnetic resonance spectroscopic imaging [MRSI]) methods. These different neuroimaging techniques have for the most part been used to study baseline neuronal characteristics or changes in correlation with symptom severity. Only a few of them have measured the effect of therapeutic interventions on brain functioning in order to explore treatment biomarkers or response predictors. It is notable that available data are not consistent and thus far, vary significantly across studies. Several factors, including sex, age, phenotype, cognitive functioning, and medication status, need to be taken into account when interpreting structural brain changes detected in GAD. Here, we briefly summarize the results of neuroimaging studies in GAD and present the key findings in *Table I* (a simplified summary).

Structural brain morphology studies

There is good evidence that GAD is characterized by significant anatomical changes in the brain, particularly within regions related to anxiety neurocircuitry. For example, increased gray matter (GM) volume in the amygdala has been repeatedly found in GAD patients.[8] Notably, increased right amygdala volume in GAD patients, mostly among females, was associated with prolonged reaction times on the tracking task, indicating attentional impairment.[9] Earlier larger volumes of the amygdala and the dorsomedial prefrontal cortex (PFC) were observed in GAD females, suggesting that these disturbances in anxiety-specific regions may be related to sex predisposition for GAD.[10]

In contrast, GM volume in the right putamen was significantly larger in GAD patients than in healthy controls, whereas a significant sex main effect was found in the left precuneus/posterior cingulate cortex, with GM volumes larger in males than in females. However, no sex-by-diagnosis interaction effect was found in this study, suggesting that GM volume in GAD is not influenced by sex.[11] The same group has also reported that a larger GM volume in the right putamen is positively correlated with childhood maltreatment.[12] A study in medication-free adolescents suffering from noncomorbid GAD reported increased GM volumes in the right precuneus and right precentral gyrus and decreased GM

Sample	Biomarker	Reference
Structural brain morphology studies		
16P (14F) 31HC (13F)	Greater GMV in Amy	Etkin et al,[8] 2009
19P (17F) 21HC (18F)	Larger right Amy	Makovac et al,[9] 2015
16P (16F) 15HC (15F)	Larger Amy and DMPFC	Schienle et al,[10] 2011
26P (13F) 25HC (12F)	Larger GMV in right Put	Liao et al,[12] 2013
15P (8F) 28HC (17F)	Increased GMV in right precuneus and precentral gyrus; decreased GMV in left OG and PC	Strawn et al,[13] 2013
19P (16F) 24HC (17F)	Higher GMV in BG; lower WMV in DLPFC	Hilbert et al,[15] 2015
20P (7F) 20HC (7F)	Reduced GM in MB, Thal, Hip, insula, STG; reduced WMV in MB, ALIC, DLPFC, PrG	Moon et al,[16] 2017
14P (8F) 10HC (6F)	Decreased volume in Hip	Abdallah et al,[18] 2013
Functional MRI studies		
17P (6F) 12HC (6F)	Greater BOLD to masked angry faces in right Amy	Mink et al,[21] 2008
15P (7F) 20HC (11F)	Greater BOLD to facial-threat cues in Amy, vPFC, ACC	McClure et al,[22] 2007
14P (12F) 12HC (10F)	Greater BOLD to both aversive and neutral pictures in bilateral dorsal Amy; higher BOLD in ACC predicts better response to treatment	Nitschke et al,[24] 2009
17P (11F) 24HC (18F)	Abnormal mPFC response to emotional conflict; absent FC in PGC-Amy	Etkin et al,[25] 2010
15P (12F) 15HC (9F)	Decreased BOLD in Amy, but increased in BNST during gambling task	Yassa et al,[27] 2012
17P (11F) 17HC (8F)	Decreased BOLD to fearful faces in Amy, but increased to angry in MFG	Blair et al,[28] 2008
8P (5F) 12HC (6F)	Increased BOLD to worry in ACC and PFC	Paulesu et al,[29] 2010
15P (15F) 16HC (16F)	Decreased BOLD to facial expressions in PFC and ACC	Palm et al,[31] 2011
17P (13F) 18HC (10F)	Decreased BOLD during explicit emotion regulation in dorsal ACC	Blair et al,[32] 2012
23P (17F) 22HC (11F)	Decreased BOLD during emotion regulation in PFC	Ball et al,[33] 2013
17P (6F) 17HC (6F)	Increased BOLD to anxiety-inducing words in VLPFC and PrG	Moon et al,[34] 2015
32P (32F) 25HC (25F)	Less discriminating BOLD response in vmPFC during safety versus threat	Cha et al,[35] 2014
32P (32F) 25HC (25F)	Hyperactivity in VTA during fear generalization task	Cha et al,[36] 2014
18P (8F) 15HC (8F)	Greater BOLD to angry faces in right VLPFC	Monk et al,[37] 2006
28P (14F) 28HC (14F)	Lower RSFC in PFC-Lim and Cin and higher in PFC-Hip	Wang et al,[40] 2016
19P (17F) 21HC (18F)	Lower RSFC between the right Amy and right SFG, right paraCin/ACC, and right SMG	Makovac et al,[41] 2016
10P (6F) 10HC (6F)	Increased BOLD in mPFC and right VLPFC	Strawn et al,[43] 2012
21P (7F) 22HC (8F)	Increased FC between Hip and FG	Cui et al,[44] 2016
26P (16F) 20HC (11F)	Abnormal FC between Amy and dPFC, Ins and STG	Liu et al,[45] 2015
15P (12F)	Greater BOLD in rACC and lower in Amy predicted better response to venlafaxine (8 wk)	Whalen et al,[46] 2008
14P (12F)	Higher BOLD in ACC predicted better response to venlafaxine (8 wk)	Nitschke et al,[47] 2009
14P (7F)	Both CBT and fluoxetine increased BOLD in right VLPFC in response to angry faces	Maslowsky et al,[48] 2010
21P (16F)	CBT decreased BOLD in Amy and subGAC in response to fear/angry faces	Fonzo et al,[49] 2014
19P (9F)	Reduced BOLD in Amy and Ins during emotional tests after acute but not 4-wk administration of alprazolam	Brown et al,[50] 2015

Table I. Summary of neuroimaging biomarkers in generalized anxiety disorder. (see abbreviations next page)

volumes in the left orbital gyrus and posterior cingulate.[13] Compared with healthy adolescents, youth with GAD exhibited increased cortical thickness in the right inferolateral and ventromedial PFC (ie, inferior frontal gyrus), the left inferior and middle temporal cortex, and the right lateral occipital cortex. No relationships were observed between cortical thickness and the severity of anxiety symptoms in the significant regions.[14] Additionally, significantly higher GM volumes were found in medication-free GAD subjects, mainly in basal ganglia structures and less consistently in the superior temporal pole; however, white matter (WM) volumes were lower in the dorsolateral PFC.[15] Similarly, significant reduction in the WM volumes in the dorsolateral PFC, anterior limb of the internal capsule (ALIC), and midbrain was observed in GAD patients who had working memory dysfunction.[16] Notably reduced dorsolateral PFC volume was negatively correlated with clinical severity and illness duration in GAD, whereas a significantly smaller orbitofrontal cortex volume was demonstrated in female than in male patients.[17]

A decrease in hippocampal volumes has also been found in GAD.[18] The distinguishable brain alternations—in particular, thinner cortices in the right medial orbitofrontal and fusiform gyri, left temporal pole, and lateral occipital regions—were found in MDD patients with comorbid GAD than in those without GAD or controls, supporting the notion that GAD is a distinct clinical entity.[19] Finally, reduced frontolimbic structural connectivity was demonstrated in patients with GAD by a diffusion-tensor imaging study, suggesting a neural basis for emotion regulation deficits in GAD.[20]

Functional MRI studies

Both neuronal response to emotional stimuli and resting-state connectivity have been investigated in a number of fMRI studies in GAD, mostly not in relation to treatment effect. The several regions traditionally connected to anxiety neurocircuitry and/or emotional regulation, including the amygdala, anterior cingulate cortex (ACC), medial PFC, ventrolateral PFC, dorsolateral PFC, and some others, have shown abnormal or changed activities in GAD. In particular, greater amygdala activation was demonstrated in pediatric patients with GAD and positively correlated with anxiety sever-

6P (3F)	Citalopram (7 wk) reduced BOLD in PFC, Str, Ins, and paraLim regions	Hoehn-Saric et al,[51] 2004
PET and SPECT studies		
7P (3F) 7HC (3F)	Unchanged 5-HTT in MB	Maron et al,[53] 2004
12P (8F) 12HC (8F)	Unchanged 5-HTT in MB, decreased DAT in Str	Lee et al,[54] 2015
10P (10F) 10HC (10F)	Decreased GABA-A in left TP	Tiihonen et al,[55] 1997
Metabolic MRI studies		
15P (8F) 15HC (8F)	Higher NAA/Cr in right DLPFC	Mathew et al,[56] 2004
9P (4F) 10HC (4F)	Lower bilateral NAA/Cr in Hip after 12 wk of paroxetin	Mathew et al,[57] 2010
15P (9F) 8HC (5F)	Increased NAA/Cr in Hip in responders to riluzole (8 wk), but decreased in nonresponders	Mathew et al,[58] 2008
15P (6F) 15HC (6F)	Lower Ch/NAA in DLPFC	Moon et al,[59] 2015

5-HTT, serotonin transporter; ACC, anterior cingulate cortex; ALIC, anterior limb of the internal capsule; Amy, amygdala; BG, basal ganglia; BNST, bed nucleus of the stria terminalis; BOLD, blood oxygen–level dependent; CBT, cognitive behavioral therapy; Ch/NAA, choline/N-acetylaspartate; Cin, cingulate; DAT, dopamine transporter; DLPFC, dorsolateral prefrontal cortex; dmPFC, dorsomedial prefrontal cortex; dPFC, doral prefrontal cortex; FC, functional connectivity; FG, fusiform gyrus; GMV, gray matter volume; Hip, hippocampus; Ins, insula; Lim, limbic; MB, midbrain; MFG, middle frontal gyrus; mPFC, medial prefrontal cortex; MRI, magnetic resonance spectroscopy; NAA/Cr, N-acetylaspartate/creatine; OG, orbital gyrus; paraCin, paracingulate; paraLim, paralimbic; PC, posterior cingulated; PET, positron emission tomography; PGC, pregenual cingulate; PrG, precentral gyrus; Put, putamen; rACC, rostal anterior cingulate cortex; RSFC, resting-state functional connectivity; SFG, superior frontal gyrus; SMG, supramarginal gyrus; SPECT, single-photon emission computed tomography; STG, superior temporal gyrus; Str, striatum; subGAC, subgenual anterior cingulate; Thal, thalamus; TP, temporal pole; VLPFC, ventrolateral prefrontal cortex; vmPFC, ventromedial prefrontal cortex; vPFC, ventral prefrontal cortex; VTA, ventral tegmental area; WM, white matter volume; wk, week

Table I. Continued

ity.[21] Earlier, other pediatric GAD studies have shown hyperactivity in the amygdala in response to negative emotional faces.[22] Also, disruptions in amygdala-based intrinsic functional connectivity networks have been reported to be similar between adult and adolescents with GAD.[23] Similar findings were also evident in adult GAD patients.[24,25] In another study, GAD patients had higher amygdala activation than healthy controls in response to neutral, but not angry, faces.[26] However, after fear induction in a gambling task, patients with GAD demonstrated decreased activity in the amygdala and increased activity in the bed nucleus of the stria terminalis when compared with controls.[27]

The involvement of cortical regions was evidenced by studies showing that in response to angry faces or triggered worry, GAD patients demonstrated increased blood oxygen–level dependent (BOLD) responses in a lateral region of the middle frontal gyrus[28] and persistent activation in both ACC and PFC areas.[29] The exaggerated early neural responses to errors, as reflected by the error-related negativity on electroencephalography (EEG), was also linked to ACC abnormalities in GAD.[30] In contrast, hypoactivation of PFC (only in female patients) or reduced dorsal ACC BOLD activity was observed in response to fearful, sad, angry, and happy facial expressions.[31,32] The BOLD hypoactivation in PFC was also demonstrated in both GAD and panic disorder during response in a reappraisal task, suggesting common neuronal pathways underlying emotion dysregulation in both anxiety disorders.[33] In contrast, significantly higher neuronal activities were observed in the ventrolateral PFC and precentral gyrus BOLD response to anxiety-inducing words.[34]

The ventromedial PFC has been shown to have a critical role in threat processing in close association with broader corticolimbic circuit abnormalities, which may synergistically contribute to GAD.[35] Moreover, the maladaptive threat processing was observed in the ventral tegmental area and the mesocorticolimbic system in female patients with GAD, which may implicate dopaminergic pathways in clinical anxiety.[36] In trials including an angry face, adolescents with GAD showed greater right ventrolateral PFC activation than healthy adolescents. This activation was negatively correlated with anxiety severity, suggesting that the neuronal increase in BOLD signal may serve as a compensatory response.[37] However, functional abnormalities in ventral cingulate and the amygdala seem to be common both for major depression and GAD, perhaps because of shared genetic factors.[38] However, those with comorbid GAD in major depression had modulated hypoactivation in response to an emotional task in middle frontal regions and the insula, as usually seen in pure depression; this gives additional support to there being different types of emotional information processing in anxiety and depression.[39]

Resting-state functional connectivity was reported to be lower in prefrontal-limbic and cingulate and higher in prefrontal-hippocampus regions, and both abnormalities were correlated with clinical symptom severity.[40] Also, the amygdala-PFC connectivity underlying worry and rumination in GAD has recently been linked to autonomic dyscontrol, suggesting overlapping neuronal substrates for cognitive and autonomic dysregulation.[41] Furthermore, amygdala and the middle frontal gyrus activation in response to presentation of emotional faces can distinguish patients with GAD and social phobia, indicating different neural circuitry dysfunctions in these two highly prevalent anxiety disorders.[28]

Previous research also implicates the ACC in emotion regulation through effects on the amygdala and suggests that deficits in ACC-amygdala connectivity may contribute to emotion dysregulation in patients with GAD.[25] Different hippocampal connectivity was observed between posttraumatic stress disorder (PTSD) and GAD patients, potentially explaining the difference in fear-related memory dysregulation in two anxiety phenotypes.[42] Increased activation of the medial PFC and right ventrolateral PFC, as well as altered connectivity between the amygdala or ventrolateral PFC and regions which subserve mentalization (eg, posterior cingulate cortex, precuneus, and medial PFC) was observed in adolescents with GAD.[43] In addition, increased functional connectivity between hippocampus/parahippocampus and fusiform gyrus was found in GAD, whereas greater functional connectivity between somatosensory cortex and thalamus was observed in panic disorder, further suggesting these two disorders have different clinical and psychopathological processes.[44] Finally, decreased functional connectivity was found between the left amygdala and left dorsolateral PFC and increased right amygdala functional connectivity with insula and superior temporal gyrus in adolescents with GAD, confirming that they have abnormalities in brain regions associated with the emotional processing pathways.[45]

Only a few fMRI studies have measured changes after treatment. The greater pretreatment reactivity to fearful faces in rostal ACC (rACC) and lesser reactivity in the amygdala have predicted a better response to 8-week treatment with venlafaxine in GAD; however, no differences between patients and controls with regard to neuronal activation within these regions were detected before treatment.[46] In addition, higher levels of pretreatment ACC activity in anticipation of both aversive and neutral pictures were associated with greater reductions in anxiety and worry symptoms after an 8-week treatment with venlafaxine in GAD.[47] This suggests that ACC-amygdala responsiveness could prove useful as a predictor of antidepressant treatment response in GAD. A significant increase in right ventrolateral PFC activation in response to angry faces after treatment with cognitive behavioral therapy (CBT) or fluoxetine was reported in small samples of young patients with GAD.[48] Greater anticipatory activity in the bilateral dorsal amygdala was shown in GAD, and a CBT course led to attenuation of amygdalar and subgenual anterior cingulate response to fearful/angry face presentation, plus heightened insular activation in response to happy faces.[49] However, the treatment had no apparent effects on increased amygdala-insular connectivity, and the changes were not associated with symptoms of worry. An interesting study with the benzodiazepine alprazolam found that neuronal activation in the amygdala and insula during emotional tests was reduced after acute administration of alprazolam. However, activity returned to baseline levels at week 4 of alprazolam treatment, indicating that the neural mechanisms supporting sustained treatment effects of benzodiazepines in GAD differ from those underlying their acute effects.[50] Significantly reduced BOLD responses to a pathology-specific worry in prefrontal regions, striatum, insula, and paralimbic regions were reported after 7 weeks of treatment with citalopram in a small sample of patients with GAD.[51]

PET and SPECT studies

Despite the great potential that radioisotope studies have to explore the neurochemical effects in live human brain, only very few have been conducted in GAD. The first PET report in GAD was by Wu et al.[52] They found lower absolute metabolic rates in basal ganglia, but found increased rates in the left inferior gyrus,

Brodmann Area (BA) 17, occipital lobe, right posterior temporal lobe, and right precentral frontal gyrus.

Unlike in MDD and other anxiety disorders, in GAD, the number of 5-HT reuptake sites available in the brain was unchanged, as measured by tracers such as [^{123}I]nor-β-CIT and [I]ADAM [53,54] In contrast, dopamine-reuptake-site density measured by [Tc] TRODAT-in the striatum was significantly lower in the GAD patients than in the healthy controls.[54] Additionally, significant decreases in the left temporal pole type A γ-aminobutyric acid (GABA-A) receptors were found in a PET study with GAD female patients.[55]

Metabolic MRI studies

The very first magnetic resonance spectroscopy study demonstrated a higher N-acetylaspartate/creatine (NAA/Cr) ratio in the right dorsolateral PFC in medication-free patients with GAD than in healthy participants.[56] The subsequent study reported persistently lower levels of bilateral hippocampal NAA/Cr in GAD after a successful 12-week treatment with paroxetine, but excluded an association between this hippocampal neuronal marker and anxiolytic response to this medication.[57] Interestingly, a significant increase in hippocampal NAA was observed in GAD responders to the glutamate-release inhibitor riluzole, whereas nonresponders had decreases over 8 weeks of treatment.[58] A low level of choline/NAA in the dorsolateral PFC was observed in GAD patients in another recent study, and this negatively correlated with anxiety severity.[59]

Genetic biomarkers

Increasing efforts are being made to determine genetic factors involved in the onset and development of psychiatric disorders and also those influencing their response to therapeutic interventions. Although several approaches have been used in this search, including epidemiological (family and twin) studies and molecular (linkage and association) methods, the genetic research for GAD is still modest compared with research undertaken for other anxiety or mood disorders. For a comprehensive discussion of the genetics of GAD, see also the article by Gottschalk and Domschke in this issue. Earlier meta-analysis of twin studies has estimated the heritability of GAD to be 32%,[60] but higher heritability estimates (49%) and no sex differences, in contrast to

previous reports, were demonstrated by a recent cross-sectional twin study in Sweden.[61]

So far, only a few association studies have been conducted among patients with the GAD phenotype, leaving us without a consistent or clear conclusion about GAD vulnerability genes. Specifically, genes for monoamine oxidase A (*MAOA*) and solute carrier family 6 member 4 (*SLC6A4*) have been implicated as potentially involved in the pathogenesis of GAD,[62,63] and the association of GAD with a 5-hydroxytryptamine receptor 1A (*5-HTR1A*) gene variation has been shown to be partly mediated by comorbidity with major depression.[64]

A recent study showed that the Met allele of the functional brain-derived neurotrophic factor (BDNF) Val66Met polymorphism is associated with GAD risk, along with an increase in serum BDNF levels.[65] However, Val66Met variation was associated with neither GAD nor BDNF plasma levels in a Chinese Han population with GAD.[66] In addition, polymorphisms both in regulator of G-protein signaling 2 (*RGS2*) and neuropeptide Y (*NPY*) genes have been shown to modify risk of post-disaster GAD under conditions of high stressor exposure among adults living in areas affected by the 2004 Florida Hurricanes[67,68] and few single-nucleotide polymorphisms (SNPs) in proteasome modulator 9 (*PSMD9*) gene were in linkage with GAD in Italian families with type 2 diabetes.[69]

Recently, the microarray study of peripheral gene expression signatures has become a powerful and promising approach in the discovery of novel biomarkers via transcriptional and microRNA analysis. For example, a microRNA (miRNA) array study performed in peripheral blood mononuclear cells (PBMCs) has revealed negative correlation between the expression level of miR-4505 and miR-663 and anxiety manifestation in GAD patients; however, the molecular mechanism of this association requires further explanation.[70] Another genome-wide peripheral gene expression study in a large sample of patients with GAD found no significant differential expression in women; however, 631 genes, most of which were immune-related, were differentially expressed between anxious and control men.[71]

Some other promising data have been reported by pharmacogenetic initiatives, where the intensive search for genetic treatment predictors has revealed a few genes, including the pituitary adenylate cyclase-activating peptide (*PACAP*), serotonin transporter (*5-HTT*), the sero-tonin 2A receptor gene (*HTR2A*), corticotropin-releasing hormone receptor 1 (*CRHR1*), dopamine receptor D3 (*DRD3*), nuclear receptor subfamily group C member 1 (*NR3C1*), and phosphodiesterase 1A (*PDE1A*), as potential markers predicting therapeutic response to SSRI medication in patients with GAD.[72-78] In contrast, none of the investigated polymorphisms within dopamine receptor D2 (DRD2) or dopamine active transporter 1 (DAT1) genes showed an impact on venlafaxine XR treatment response in GAD.[79]

Neurochemical biomarkers

Plasma appears to be a rational source for proteomic and metabolomic measurements because it is easily accessible and because several molecules from the brain are transported across the blood-brain barrier and reach the circulation. However, drawing inferences from the neurochemical composition of plasma on the processes in the brain is not straightforward.[80] Moreover, only a few studies have been conducted on plasma-based pathogenetic and/or treatment predictors in GAD, indicating the further need to explore such potentially valuable approaches. So far, the studies measuring 5-hydroxytryptamine (5-HT, also called serotonin)-related biomarkers have found decreased platelet 5-HT-reuptake-site binding in GAD patients,[81] but unchanged 5-HT binding in lymphocytes as compared with controls.[82] Moreover, GAD patients showed concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in platelet-rich and platelet-poor plasma, as well as in lymphocytes, within the normal range.[82]

Unlike for other anxiety disorders, particularly PTSD, it seems that GAD is not characterized by consistent evidence of possible abnormalities in the regulation of HPA-axis activity. Male patients with GAD displayed similar cortisol plasma levels after a stressful test.[83] A greater cortisol awakening response has been reported only for those GAD patients who had comorbid MDD.[84] The large studies performed among 4256 Vietnam-era veterans showed similar cortisol and dehydroepiandrosterone sufate (DHEAS) plasma levels and cortisol/DHEAS ratios between GAD sufferers and normal controls.[85]

Another approach is to use challenge tests to provoke anxiety/stress. In one, administration of 7.5% carbon dioxide did not significantly change salivary cortisol levels in medication-free GAD patients.[86] Moreover,

no difference in pre-sleep salivary cortisol level was found among children with GAD, despite the presence of sleep disturbances.[87] On the other hand, both higher and lower cortisol awakening responses were observed among elderly GAD patients than with nonanxious controls, positively associated with symptomatic severity in one study[88] and irrespective of the duration of illness in another one.[89] Furthermore, both untreated and venlafaxine-treated GAD patients demonstrated significantly higher cortisol levels than normal controls in a clonidine-challenge study.[90] Nevertheless, some studies reported a significant reduction in post-treatment cortisol levels after successful psychological or pharmacological treatment of GAD. For example, elevated plasma cortisol levels decreased after successful CBT,[91] and greater reductions in both peak and total salivary cortisol were shown in elderly GAD patients after escitalopram treatment than in placebo-treated patients.[92] However, no association was reported between a positive therapeutic outcome to buspirone[93] or alprazolam[94] and post-treatment cortisol levels in GAD.

Although a strong link between neurotrophic factors and mood disorders is well established, it seems that this relationship in GAD is not so obvious or may have the opposite effect.[80] No changes in BDNF levels were found in a sufficiently large sample of patients with different anxiety disorders, including GAD.[95] However, a small study comparing patients with GAD or MDD with healthy subjects showed doubled plasma levels of BDNF and artemin, a glial-cell-line–derived neurotrophic factor family member, in GAD patients compared with normal controls, whereas depressed patients showed a reduction.[96] The baseline plasma BDNF levels were not associated with GAD severity; however, a significantly greater mean increase in plasma BDNF level was observed in duloxetine-treated patients than in those who had received placebo.[97] Interestingly, an increased plasma concentration of nerve growth factor was observed among GAD patients after successful CBT.[98]

Finally, among immunological factors, C-reactive protein (CRP) was found to be elevated in some studies.[99,100] A pilot study that measured peripheral levels of cytokines in small cohorts of GAD and MDD patients has demonstrated an increase in plasma concentrations of interleukin (IL)-10 and α-melanocyte-stimulating hormone (α-MSH), but no significant variations in IL-2.[101] Earlier, a study in patients with GAD and panic disorder with agoraphobia measured cell-mediated immune functions through the lymphocyte proliferative response to phytohemagglutinin, IL-2 production, and natural killer cell activity and suggested a reduction in this function when compared with healthy controls.[102]

Conclusion

GAD is still something of an orphan disorder in terms of known biomarkers, as well as in the diagnosis of anxiety disorders. To some extent, this is due to the marked and common overlap of GAD with major depression; also, because the severity of the illness' impact on the activities of the patients is often overlooked, research funds are limited. However, as this review shows, there are meaningful differences in the biology of GAD and depression that not only help confirm that these are indeed separate diseases but that they may in some cases be polar opposites. This may help explain the well-established phenomenon that GAD often precedes depression, and the emergence of depression may represent a failure of the compensatory activities that the body mounts to protect itself against the chronic stress imposed by GAD. Such differences have significant implications for improving diagnosis and thus enhance the understanding and treatment of GAD as a different illness from its better-recognized cousins, such as panic disorder and obsessive-compulsive disorder. Also, new biological insights have major implications for developing new treatments. These are sorely needed, as there has been no major advance in the treatment of GAD for many years, and thus, patients continue to suffer. If we could better target the few treatments we have on the basis of biomarkers, then we could at least work to optimize patient response to medication. ❑

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Marcadores biológicos del trastorno de ansiedad generalizada

El trastorno de ansiedad generalizada (TAG) es una condición de salud mental prevalente y muy discapacitante; sin embargo, aún hay mucho que aprender en relación con biomarcadores específicos, como también respecto del diagnóstico, lo que se dificulta más por la sobreposición marcada y frecuente del TAG con los trastornos afectivos y de ansiedad. Recientemente, el TAG ha sido objeto de grandes esfuerzos de investigación, mediante la aplicación de neuroimágenes, estudios genéticos y exámenes sanguíneos enfocados en el descubrimiento de biomarcadores relacionados con la patogenética y el tratamiento. Este artículo revisa la gran cantidad de información disponible y se enfoca especialmente en la evidencia que proviene de los resultados de las neuroimágenes, la genética y las mediciones neuroquímicas en el TAG, con el objetivo de tener una mejor comprensión de los potenciales biomarcadores involucrados en su etiología y terapéutica. En general, la mayoría de estos estudios ha entregado resultados que constituyen hallazgos aislados, algunas veces inconsistentes y no claramente replicables. Por estas razones, estos resultados aún no se han traducido en la práctica clínica. Por consiguiente, se necesitan más esfuerzos de investigación para distinguir el TAG de otros trastornos mentales y contar con nuevos hallazgos biológicos para su patogenia y tratamiento.

Les marqueurs biologiques de l'anxiété généralisée

L'anxiété généralisée (AG) est un trouble de santé mentale prévalent et très invalidant. Il reste cependant beaucoup à apprendre sur des biomarqueurs pertinents ainsi que sur le diagnostic, rendu plus difficile par le chevauchement important et courant de l'AG avec les troubles affectifs et anxieux. Récemment, l'AG a fait l'objet d'efforts intenses de recherche, appliquant la neuro-imagerie, la génétique et les analyses sanguines à la découverte de biomarqueurs pathogènes et liés au traitement. Dans cet article, nous analysons l'important volume de données disponibles et nous nous concentrons en particulier sur des données de neuro-imagerie, de génétique et des mesures neurochimiques dans l'AG, afin de mieux comprendre les biomarqueurs potentiels impliqués dans son étiologie et son traitement. Globalement, la majorité de ces études sort des résultats isolés, parfois contradictoires et non clairement reproductibles. C'est pourquoi ils n'ont toujours pas été transposés en pratique clinique. Il faut donc d'autres efforts de recherche pour différentier l'AG des autres troubles mentaux et permettre de nouvelles découvertes biologiques dans sa pathogenèse et son traitement.